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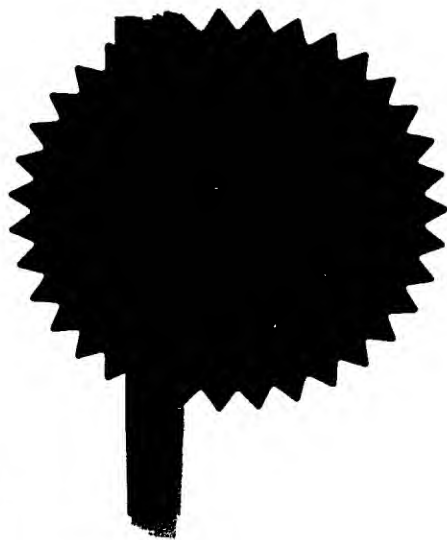
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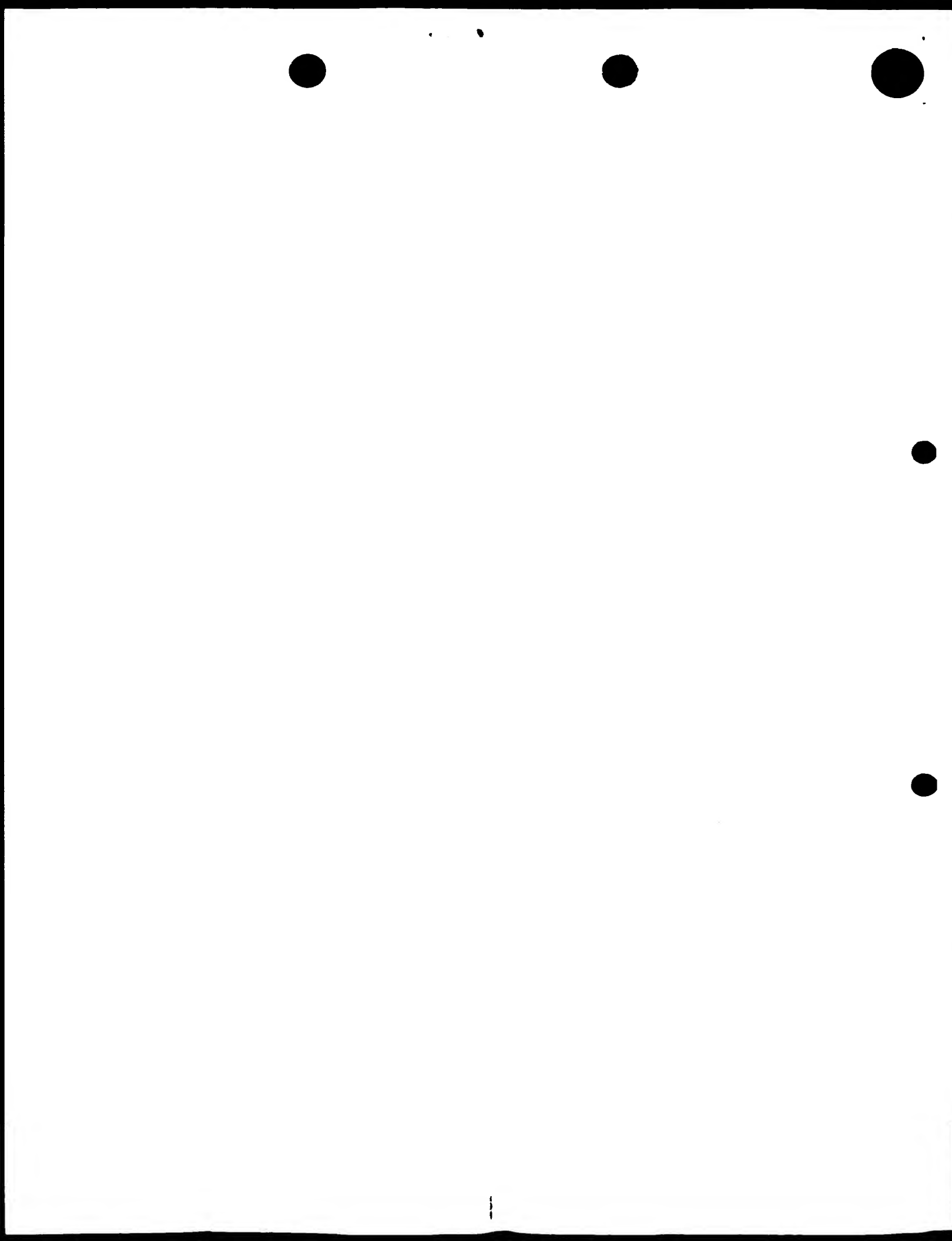
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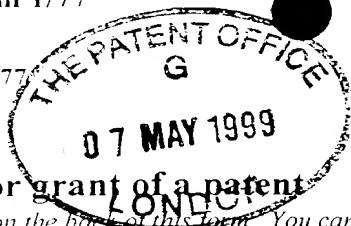
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1.	Your reference	JDM/DCS/P.400063GB		
2.	Patent application number (The Patent Office will fill in this part)	07 MAY 1999	9910693.2	
3.	Full name, address and postcode of the or of each applicant (<i>underline all surnames</i>)	THE UNIVERSITY OF LIVERPOOL, Senate House, Abercromby Square, Liverpool. L69 3BX.		
	Patents ADP number (<i>if you know it</i>)			
	If the applicant is a corporate body, give the country state of its incorporation	798 355 001 nides		
4.	Title of the invention	A COMPOUND FOR USE IN MEDICINE		
5.	Name of your agent (<i>if you have one</i>)	W.P.THOMPSON & CO.		
	"Address for service" in the United Kingdom to which all correspondence should be sent (<i>including the postcode</i>)	Coopers Building, Church Street, Liverpool. L1 3AB.		
	Patents ADP number (<i>if you know it</i>)	0000158001 ✓		
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (<i>if you know it</i>) the or each application number	Country	Priority application number (<i>if you know it</i>)	Date of filing (<i>Day/month/year</i>)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (<i>Day/month/year</i>)
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? (<i>Answer 'yes' if:</i> a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body See note (d))			

Patents Form 1/77

9. Enter then number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description 8

Claims(s)

Abstract

Drawing(s) 1 + 1

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*)

Request for substantive examination (*Patents Form 10/77*)

Any other documents
(Please specify)

11. I/We request the grant of a patent on the basis of this application

Signature Date

W.P. THOMPSON & CO. 7th May 1999

12. Name and daytime telephone number of person to contact in the United Kingdom

Dominic C. Schiller - 0151-709-3961

JDm/DCS/P. 400063 GtB

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DESCRIPTIONA COMPOUND FOR USE IN MEDICINE

The present invention relates to the use of a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein, more particularly an osteoclast associated ABC protein, for use in medicine, and more particularly to the use of a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein, more particularly an osteoclast associated ABC protein for use in the manufacture of a medicament for use in the treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease. Such diseases include, but are not limited to, osteoporosis, Paget's disease, bone metastases, myeloma and humoral hypercalcaemia of malignancy.

The invention also relates to a method of screening for a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein, more particularly an osteoclast associated ABC protein, comprising determining whether the presence of said compound leads to full or partial inhibition of bone resorption.

ABC proteins (ATP binding cassette proteins), also called traffic ATP ases, are a super family of transmembrane proteins involved in the movement of substrates across cell membranes. ABC proteins are abundant in prokaryotes where they represent almost 5% of the total genome and show specificity for a diverse range of

substrates ranging from peptides and amino acids to ions and sugars. ABC proteins are characterised by the presence of 2 peptide motifs, Walker A and Walker B motifs. These motifs are common to many nucleotide binding proteins. However, ABC proteins are distinguished from these other proteins by the presence of a third C-signature motif, separating Walker A and B motifs with conserved spacing.

The importance of ABC proteins in mammalian systems is now being recognised. Several members of the ABC family have been shown to be important in human disease, their dysfunction results in a variety of disease states including cystic fibrosis, multi-drug resistance of tumour cells, non-insulin dependent diabetes and adrenoleukodystrophy. These ABC proteins are known to be involved in the translocation of ion and hydrophobic drugs across the plasma membrane. Other human ABC proteins have also been shown to be involved in peptide translocation (PAB) and phospholipid transfer across the canalicular membrane, as is the case with the MRP sub family. In addition, human ABC - 1 has recently been implicated as a regulator of phospholipid equilibrium and non-classical (signal independent) secretion of IL1- β in macrophages.

The applicant has discovered ABC transporter proteins in bone and osteoclast rich tissue and identified several novel members of the ABC protein family from osteoclastoma cDNA libraries and human bones cDNA libraries by immunoscreening and hybridisation screening.

The applicant's discovery suggests compounds which will either inhibit or promote expression or function of an ABC protein, more particularly an osteoclast

associated ABC protein, may be useful in treating conditions arising out of osteoclastic function.

The role of ABC proteins, more particularly an osteoclast specific protein has not previously been identified.

However, in view of the fact that P-glycoprotein has recently been found to be present in osteoblasts (Calcified Tissue International, 1996 Vol 58 No. 3 P186-191) one can postulate that members of the ABC family of proteins may be involved in bone formation.

More generally, ATP binding cassette proteins have been implicated in many cellular functions. As such an osteoclast associated family of ABC transporters may regulate many processes within these cells.

The ability of members of the ABC super family to regulate volume-activated channels via ATP release has been documented in other cell types.

Osteoclasts are terminally differentiated cells and are hence programmed to die by apoptosis. The ABC1 member of the ABC superfamily has been implicated in the recognition of apoptotic cells by macrophages, a process thought to involve transmembrane flux of phosphatidylserine.

Inhibition or promotion of some of these putative functions of ABC proteins expressed in bone and osteoclasts could have complex effects on, for example, bone resorption ranging from inhibition, through no effect, to stimulation. Inhibition of osteoclast apoptosis, would lead to a subsequent elevation in the functional osteoclast pool and enhanced resorption. Similarly, sulphonylurea sensitivity is conferred on

K/ATP channels through the presence of ABC transporters, inhibition of which blocks potassium ion efflux and consequent calcium ion influx thereby promoting insulin secretion. It is therefore possible that blocking osteoclast associated ABC transporters associated with ion channels may enhance release of factors that may stimulate resorption, including regulatory factors, protons and proteases including Cathepsin K. Conversely, the processes of osteoclast fusion from mononuclear precursors, and those of cellular adhesion, if blocked will lead to decreased resorption. As suggested earlier, transmembrane phospholipid trafficking by the ABC1 transporter provides a mechanism for osteoclast fusion, whilst cellular adhesion may be promoted by annexin-mediated binding between phospholipids and extracellular matrix.

The applicant has gone on to convincingly demonstrate that inhibition of ABC proteins using Glibenclamide, a known inhibitor of known ABC proteins, in osteoclast containing populations inhibits resorption. These results demonstrate that inhibitors of ABC proteins will be useful therapeutic agents in the therapy of diseases where inhibition of resorption is desirable. These include osteoporosis, Paget's disease, bone metastases, myeloma and humoral hypercalcaemia of malignancy.

According to a first aspect of the present invention there is provided a compound which will act as an inhibitor or antagonist of an ABC protein for use in the manufacture of a medicament for use in the treatment of a disease where full or partial inhibition of bone resorption will result in an improvement in the disease.

Examples of existing compounds which have similar action to the sulphonyl

urea, Glibenclamide, include:

TOLBUTAMIDE,
CHLOROPROPAMIDE,
TOLOZAMIDE,
GLIPIZIDE,
GLIQUIDONE, and
GLICLAZIDE.

The compounds may be administered orally, intravenously, or by any other traditional route. The preferred adult dosage, for oral application, ranges from 0.001g to 5g daily, more preferably 0.01g to 0.5g daily.

This discovery also allows candidate compounds to be screened.

According to a further aspect of the present invention there is provided a method of screening for a compound which will act as an inhibitor or antagonist of the expression or function of an ABC protein comprising determining whether the presence of said compound leads to full or partial inhibition of bone resorption.

The invention will be further described, by way of example only, with reference to the following test data.

Identification of novel ABC transporters from human giant cell tumour

Monoclonal antibodies were raised against cells from human bone. One antibody was shown to strongly stain the osteoclast. In order to identify the antigen a human bone cDNA expression library constructed in lambda gt11 was

immunoscreened. Two clones were identified of size 300bp and 435bp. A second separate immunoscreen identified the 435bp clone which was then sequenced and identified as a partial length cDNA clone encoding a novel ATP binding cassette (ABC) protein. Subsequently this clone was used to hybridization screen an osteoclastoma cDNA library in lambda gt11 and 19 clones were identified and purified. The longest sequenced was 1.5kb and found to be a highly homologous, but distinct from the 435bp clone and encoded a second novel ABC transporter. Further sequencing of the additional clones suggests that there may be additional family members. Comparative sequence analysis and phylogenetic analysis demonstrates that these ABC's comprise a novel sub family of transporters which have not previously been described. This novel sub family of ABC transporters would appear to have restricted tissue expression.

Inhibition of resorption by Glibenclamide.

In order to determine whether ABC proteins were involved in bone resorption the applicant conducted in vitro bone resorption assays (as per Methods in Molecular Medicine, Human cell culture protocols, ed G.E. Jones, Humana Press, pages 263-275) to look at the effect of a well characterised ABC protein inhibitor, Glibenclamide, using avian and human osteoclasts.

The results are shown in figures 1 to 3. Figure 1 shows inhibition of resorption by Glibenclamide using avian osteoclasts in vitro;

Figure 2 shows inhibition of resorption by Glibenclamide using human osteoclasts derived from a giant cell tumor;

Figure 3 shows the dose response as inhibition of resorption by Glibenclamide using human osteoclasts.

The vehicle is tissue culture medium containing 0.1% dimethyl sulphoxide.

In summary Glibenclamide at concentrations of 100 micromolar inhibited resorption by settled suspensions of avian bone cells (figure 1). These findings were mirrored using a suspension of giant cells obtained from a human osteoclastoma or giant cell tumour (figure 2). Glibenclamide at concentrations lower than 50 micromolar did not inhibit resorption, however, at concentrations of 50 and 100 micromolar resorption was depressed (figure 3). This was confirmed using a different giant cell population. This data demonstrates inhibiting ABC proteins inhibits bone resorption.

More details of the procedure followed are given below.

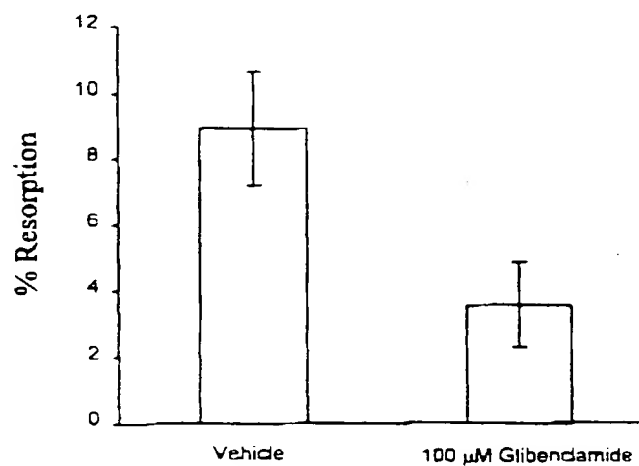
Osteoclasts were dislodged from human giant cell tumour by agitation in α MEM. Sterile devitalised bone wafers were placed in a culture dish and the GCT suspension dripped over them using a sterile 10ml syringe. The culture was then incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO₂ for 20 min.

Wafers were then removed and washed in PBS to dislodge any non-adherent cells. Wafers were then transferred to 24 well plates, each containing 900 μ l of α MEM supplemented with 10% foetal calf serum and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO₂ for 24 hours. Cells were treated by adding 100 μ l of 10x concentration of inhibitor and incubated at 37°C for a further 72 hours as described above. Bone wafers were then washed in PBS at 37°C, fixed in 4%

glutaraldehyde in 0.2% sodium cacodylate, and stained for 5 min in 1% (w/v) toluidine blue in 0.5% disodium tetraborate. Resorption lacunae present on stained devitalised bone wafers were identified using an Olympus BH2 microscope fitted for incident light microscopy with metallurgic objectives. The plan area of resorption was determined by point counting using a 10X objective and drawing tube and expressed as a percentage of the total plan area of the bone wafers.

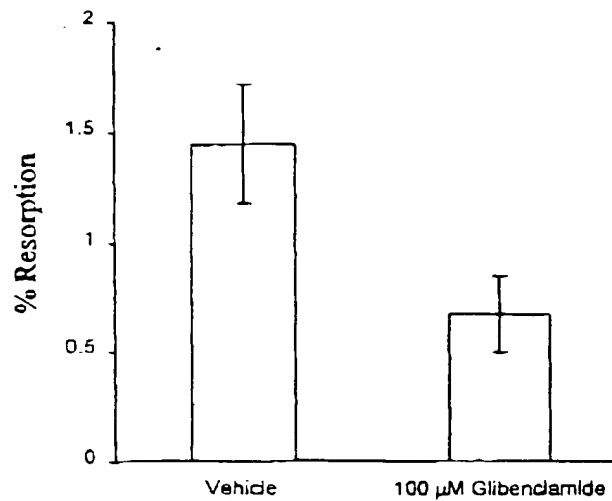
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Figure 1



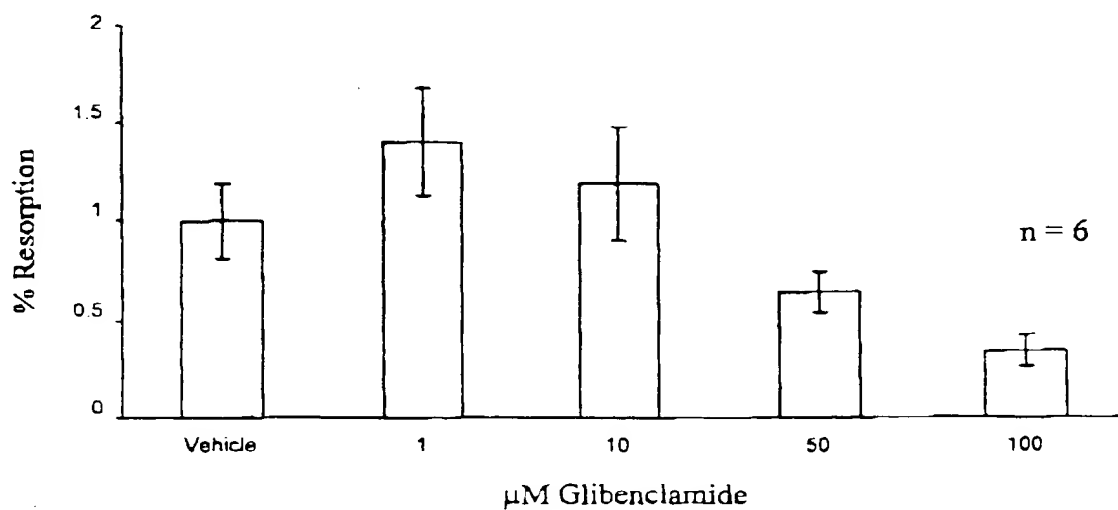
n = 6

Figure 2



n = 7

Figure 3



n = 6

General

Account

5/5/00

Agent

WP

Thompson

L. Co